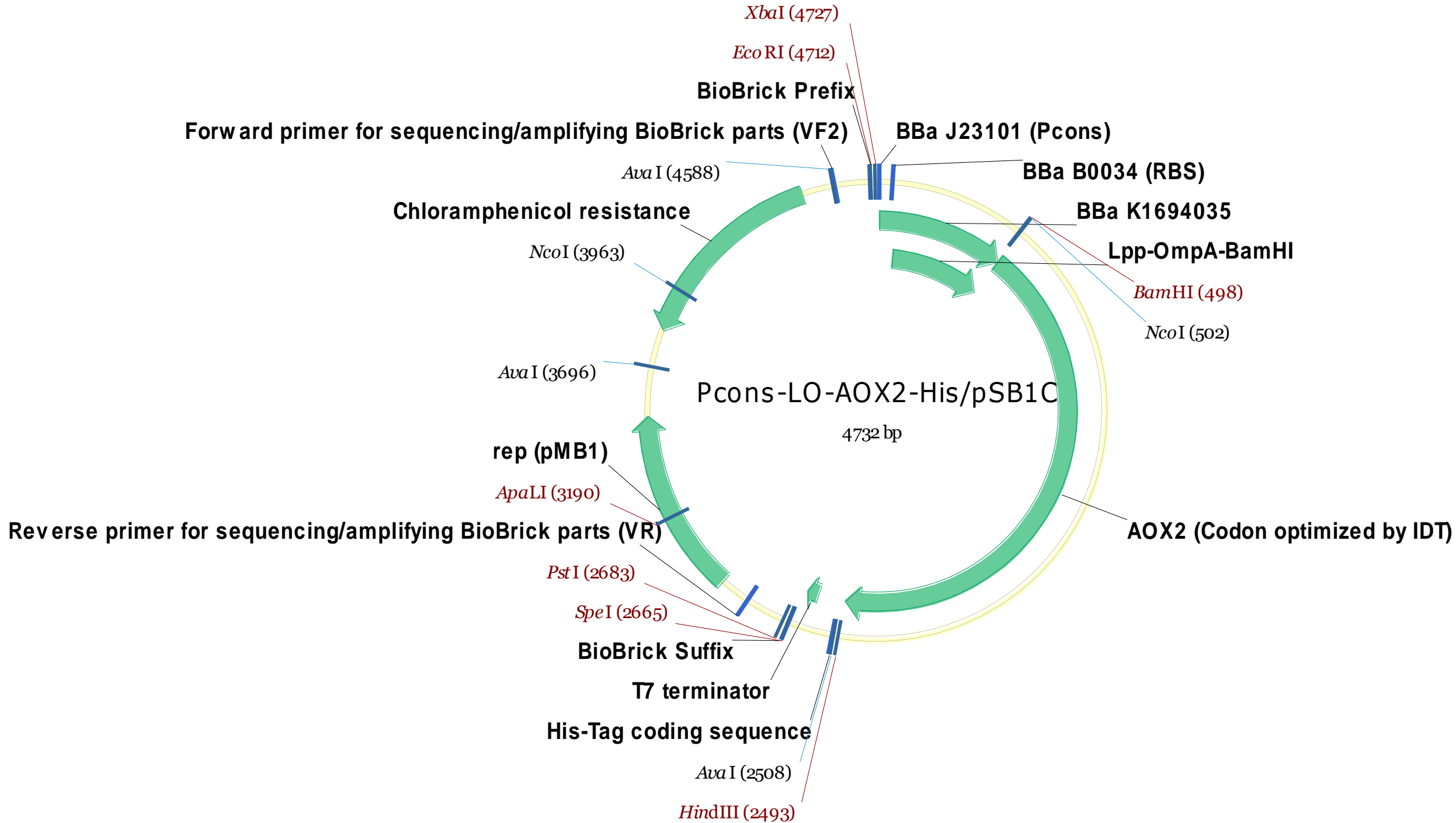


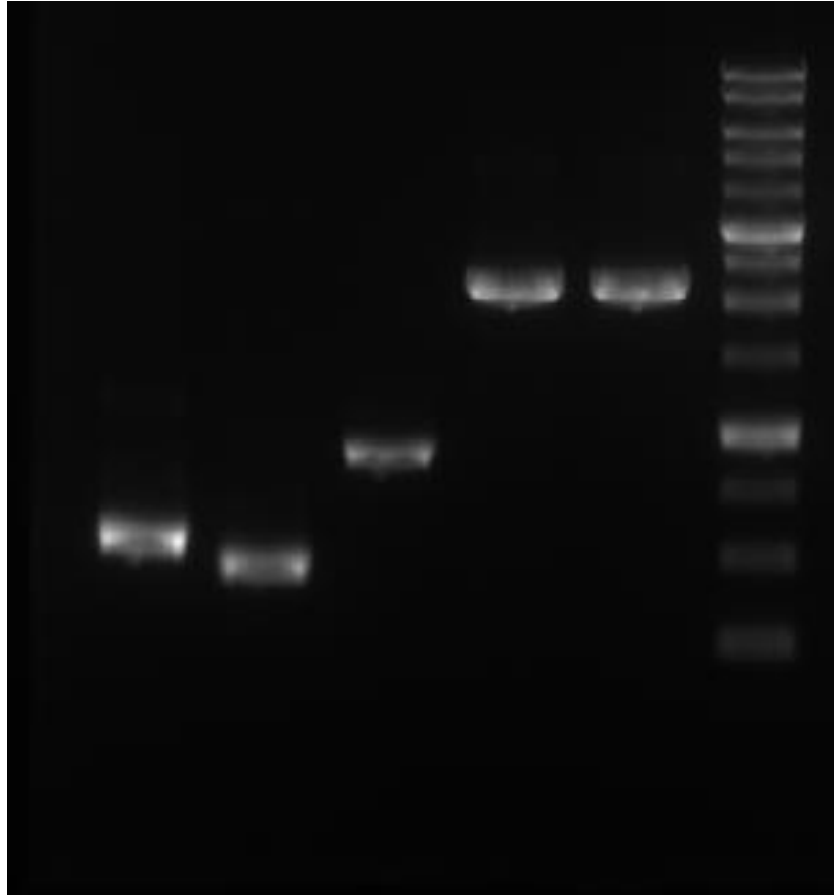
**Pcons-RBS-LO-AOX2-  
His/pSB1C3**



# Strategy

- The same as “CLONING - LO-AOX2-His\_pSB1C3”

# PCR with KOD-plus



- Date: 2016.08.17

- Lane:

1. Pcons-RBS-LO-BamHI

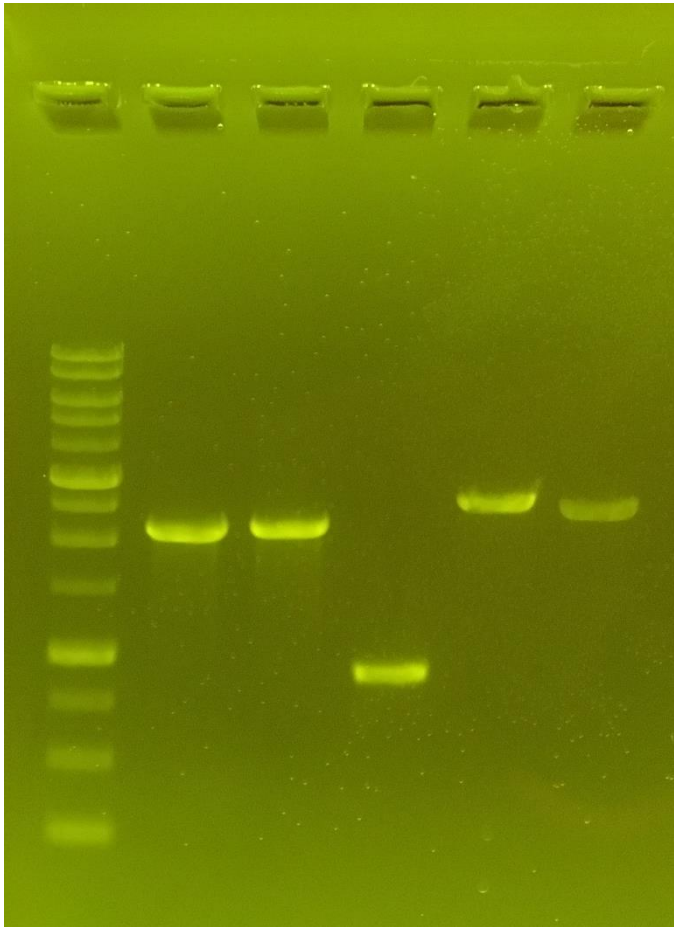
2. LO-BamHI

3. GFP

4. AOX1

5. AOX2

# Ligation



- Date: 2016.08.31

- Lane:

1. AOX1: BamHI + PstI

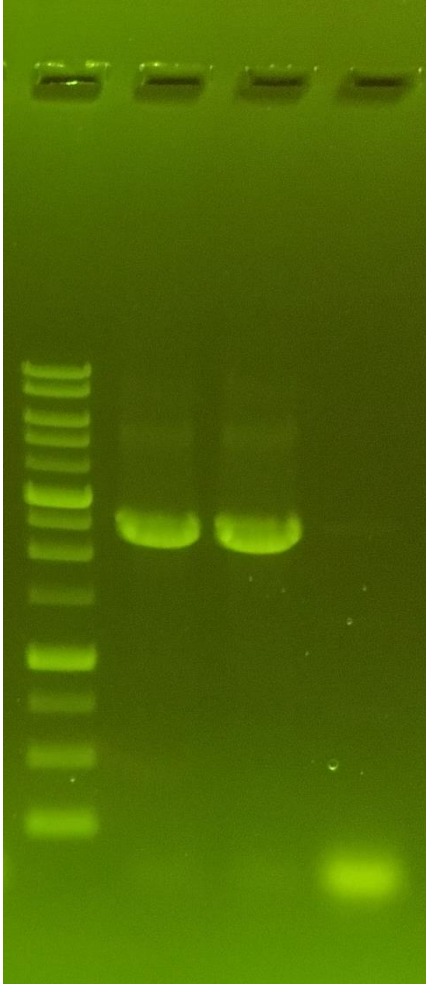
2. AOX2: BamHI + PstI

3. GFP: BamHI + PstI

4. Pcons-RBS-LO-BamHI/pSB1C3  
#P6: BamHI + PstI

5. LO-BamHI/pSB1C3 #L2: BamHI  
+ PstI

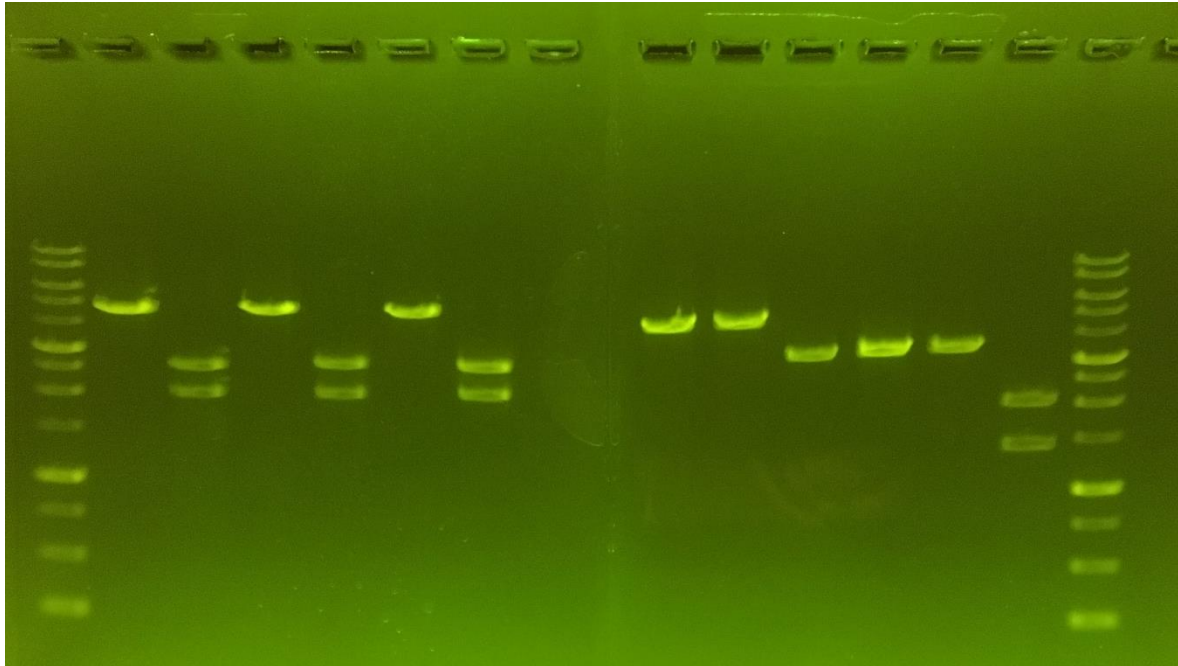
# Colony PCR



- Date: 2016.09.02
- Clone: Pcons-RBS-LO-AOX2-His/pSB1C3
- Primer:
  1. Pcons-Xbal-EcoRI-F
  2. AOX2-PstI-R
- Lane: #P2-1 ~ #P2-3
- #P2-2 → Plasmid extraction → RE check

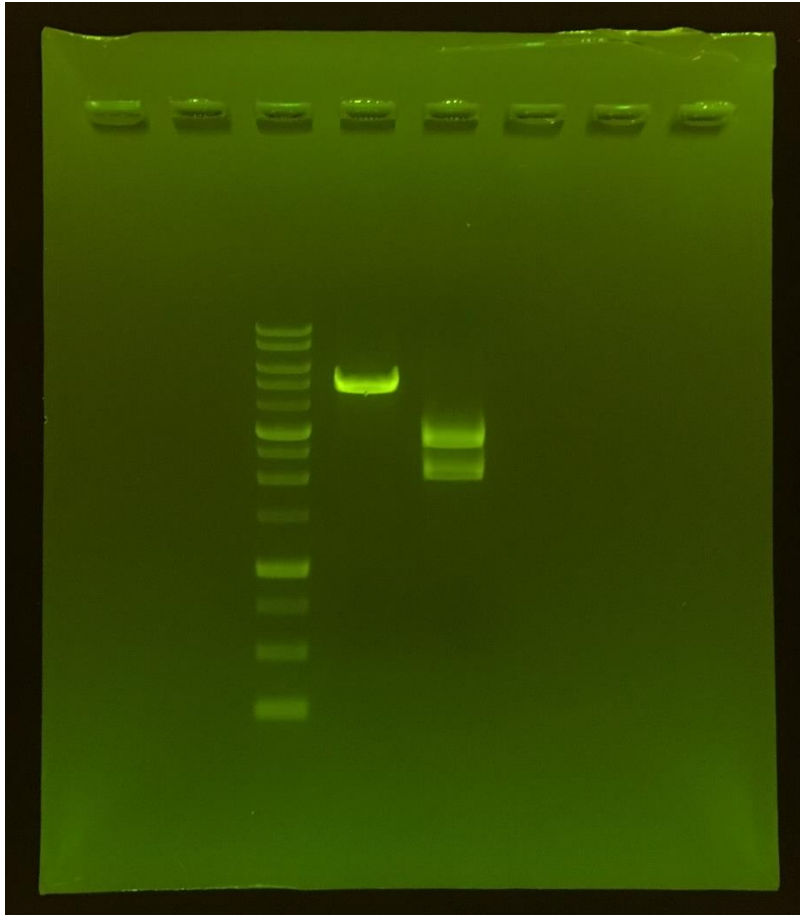
# RE check

- Date: 2016.09.07
- Clone: Pcons-RBS-LO-AOX2-His/pSB1C3
- Lane:



1. #L1-2: EcoRI
2. #L1-2: EcoRI + PstI
3. #P1-5: EcoRI
4. #P1-5: EcoRI + PstI
5. #L2-5: EcoRI
6. #L2-5: EcoRI + PstI
7. #P2-2: EcoRI (Failed)
8. #P2-2: EcoRI + PstI (Failed)
9. #LG-3: EcoRI (Failed)
10. #LG-3: EcoRI + PstI (Failed)
11. #PG-4: EcoRI
12. #PG-4: EcoRI + PstI

# RE check II



- Date: 2016.09.08
- Clone: LO-AOX2-His/pSB1C3
- Lane:
  1. #P2-1: EcoRI
  2. #P2-1: EcoRI + PstI
- Sequencing



# Sequencing

- Date: 2016.09.14
- Sequencing primers: VF2, VR
- Clone: Pcons-RBS-LO-AOX2-His/pSB1C3 **#P2-1**
- File: “2016.09.14\_Pcons-RBS-AOX2\_pSB1C3 (#P2-1)”
- Result: correct → make a stock